(FILE 'HOME' ENTERED AT 20:45:35 ON 26 NOV 2001)

FILE 'MEDLINE, BIOSIS, CANCERLIT, LIFESCI' ENTERED AT 20:46:11 ON 26 NOV 2001

```
L1         74440 S (T(W)CELL(2W)RECEPTOR#) OR TCR
L2          2564 S L1(S)ZETA
L3           472 S L2(S)(RECOMBINANT OR ENGINEER? OR FUSION# OR CHIMER?)
L4           156 S L3(S)ANTIBOD?
L5            63 S L4 AND PY<1996
L6            22 DUP REM L5 (41 DUPLICATES REMOVED)
L7         38123 S ANTIBOD?(S)(FUSION OR CHIMER?)
L8           412 S L7(S)(SPACER OR HINGE)
L9           210 S L8 AND PY<1996
L10           87 DUP REM L9 (123 DUPLICATES REMOVED)
L11           51 S L7 AND (SPACER OR HINGE)/TI
L12           35 S L11 AND PY<1996
L13           14 DUP REM L12 (21 DUPLICATES REMOVED)
L14         1735 S SINGLE CHAIN? ANTIBOD?
L15         5367 S SFV OR SCFV
L16           60 S L14(S)(SPACER OR HINGE)
L17          160 S L15(S)(SPACER OR HINGE)
L18          171 S L16 OR L17
L19           44 S L18 AND PY<1996
L20           17 DUP REM L19 (27 DUPLICATES REMOVED)
L21           17 S L14(S)(LINKERS)
L22           80 S L15(S)(LINKERS)
L23           87 S L21 OR L22
L24           32 S L23 AND PY<1996
L25           14 DUP REM L24 (18 DUPLICATES REMOVED)

=> log h
COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
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DB Name	Query	Hit Count	Set Name
JPAB,EPAB,DWPI	l17 with zeta	13	L18
JPAB,EPAB,DWPI	(t adj cell adj2 receptor$1) or TCR	1366	L17
USPT	l15 not l14	11	L16
USPT	l13 and @prad<19950420	16	L15
USPT	l13 and @ad<19950420	69	L14
USPT	l12 and ((spacer$1 or hinge$1 or linker$1) with (require$4 or necessary or enhanc$4))	236	L13
USPT	(single adj chain$2 adj antibod$3) or scFv or sFV	3602	L12
USPT	l8 and spacer	0	L11
USPT	l8 and linker	1	L10
USPT	l8 and hinge	0	L9
USPT	5888773[pn]	1	L8
USPT	588873[pn]	0	L7
USPT	l4 and @prad<19950420	0	L6
USPT	l4 and @ad<19950420	7	L5
USPT	l3 same (recombinant or engineered or fusion or chimer$2)	15	L4
USPT	l2 same ((antigen adj binding adj region) or antibod$3)	41	L3
USPT	l1 with zeta	94	L2
USPT	(t adj cell adj2 receptor$1) or TCR	4154	L1

L8 ANSWER 1 OF 2 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 1
ACCESSION NUMBER: 96:734007 SCISEARCH
THE GENUINE ARTICLE: VL106
TITLE: MULTIVALENT ANTIBODY FRAGMENTS WITH HIGH FUNCTIONAL AFFINITY FOR A TUMOR-ASSOCIATED CARBOHYDRATE ANTIGEN
AUTHOR: RHEINNECKER M; HARDT C; ILAG L L; KUFER P; GRUBER R; HOESS A; LUPAS A; ROTTENBERGER C; PLUCKTHUN A; PACK P (Reprint)
CORPORATE SOURCE: MORPHOSYS GMBH, FRANKFURTER RING 193A, D-80807 MUNICH, GERMANY (Reprint); MORPHOSYS GMBH, D-80807 MUNICH, GERMANY; INST IMMUNOL, MUNICH, GERMANY; MAX PLANCK INST BIOCHEM, D-8033 MARTINSRIED, GERMANY; UNIV ZURICH, INST BIOCHEM, ZURICH, SWITZERLAND
COUNTRY OF AUTHOR: GERMANY; SWITZERLAND
SOURCE: JOURNAL OF IMMUNOLOGY, (01 OCT 1996) Vol. 157, No. 7, pp. 2989-2997.
ISSN: 0022-1767.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 57

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We report in this work a human-derived self-assembling polypeptide based on the tetramerization domain of the human transcription factor p53, which can be fused to single-chain Fv Ab (scFv) fragments via a long and **flexible** hinge sequence of human origin, allowing exploitation of the functional **affinity** increase of binding to a ligand or cell surface with multimeric binding sites, We have demonstrated the use of this polypeptide by applying it to the construction of a tetrameric scFv against the tumor-associated carbohydrate Ag Lewis Y (Fuc alpha(1)-->2Gal beta(1)-->4[Fuc alpha(1)-->3] GlcNAc beta(1)-->3R). For comparison purposes, the corresponding scFv and dimeric mini-antibody, comprising the scFv fused via a **flexible** murine hinge to an artificial dimerization domain, were also created, The recombinant mini-antibody proteins were expressed in functional form in Escherichia coli and showed the expected m.w. of a dimer and tetramer, respectively, Analysis of Lewis Y-binding behavior by surface plasmon resonance revealed specific but very weak binding of the scFv fragment. In contrast, both dimeric and tetrameric **scFv fusion** proteins exhibited an enormous gain in functional **affinity** that was greatest in the case of the tetrameric mini-antibody.

L13 ANSWER 4 OF 14 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 93294295 MEDLINE
DOCUMENT NUMBER: 93294295 PubMed ID: 7685795
TITLE: Studies on antigen binding by intact and **hinge**-deleted **chimeric antibodies**.
AUTHOR: Horgan C; Brown K; Pincus S H
CORPORATE SOURCE: Laboratory of Microbial Structure and Function, Rocky Mountain Laboratories, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT 59840.
SOURCE: JOURNAL OF IMMUNOLOGY, **(1993 Jun 15)** 150 (12) 5400-7.
Journal code: IFB; 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199307
ENTRY DATE: Entered STN: 19930806
Last Updated on STN: 19960129
Entered Medline: 19930720

AB A matched set of **chimeric** IgG1 and IgG4 **antibodies** were used to investigate the role of the IgG hinge in binding to Ag with differing space between the epitopes. **Antibodies** bearing identical V regions and either IgG1 or IgG4 C regions were engineered with and without hinges. We measured the binding of these **antibodies** to the peptide CYYYEEEEY and to CYYYEEEEY-BSA conjugates with decreasing numbers of peptides per BSA molecule. We earlier showed that V region differences in **antibodies** could affect Ag binding patterns in solid-phase but not solution-phase assays; however, both types of assay yielded similar results for the hinge-deleted **antibodies**. Binding of CYYYEEEEY-BSA by hinge-deleted and intact IgG1 was similar, but intact IgG1 bound free peptide better than did hinge-deleted IgG1. Intact IgG4 **antibody** bound less well to CYYYEEEEY and CYYYEEEEY-BSA than did IgG1 but, surprisingly, hinge-deleted IgG4 showed better binding than did intact IgG4 and was more like the IgG1 **antibodies** in binding affinity. Thus, the IgG4 hinge may impart a structural constraint that prevents high affinity binding to Ag. The hinge-deleted IgG4 **antibody** did not activate C, although it bound Ag similarly to IgG1. This study is the first to address the effect of the IgG hinge on Ag binding by using well defined Ag with different epitope densities. Our results may provide an explanation for the apparent low affinity of IgG4 **antibodies**.

L13 ANSWER 5 OF 14 MEDLINE DUPLICATE 3

WEST

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L18: Entry 10 of 13 File: DWPI Dec 1, 1998

DERWENT-ACC-NO: 1999-044582
DERWENT-WEEK: 199930

TITLE: Membrane-bound chimeric receptors - comprising extracellular portion which recognises and binds a target cell and an intracellular portion of e.g. a T-cell receptor

INVENTOR: KOLANUS, W; ROMEO, C ; SEED, B

PATENT-ASSIGNEE: GEN HOSPITAL CORP (GEHO)

PRIORITY-DATA: 1992US-0847566 (March 6, 1992), 1991US-0665961 (March 7, 1991), 1994US-0203866 (February 28, 1994), 1995US-0417495 (April 5, 1995)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 5843728 A	December 1, 1998		057	C12P021/02

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
US 5843728A	March 7, 1991	1991US-0665961	CIP of
US 5843728A	March 6, 1992	1992US-0847566	Cont of
US 5843728A	February 28, 1994	1994US-0203866	Cont of
US 5843728A	April 5, 1995	1995US-0417495	

INT-CL (IPC): C07H 17/00; C07K 14/705; C12N 5/22; C12P 21/02

RELATED-ACC-NO: 1992-331474;1995-067330 ;1995-292893 ;1996-129034 ;1996-402358

ABSTRACTED-PUB-NO: US 5843728A
BASIC-ABSTRACT:

DNA (I) encoding a membrane-bound chimeric receptor comprises (a) an extracellular portion that specifically recognises and binds a target cell or a target infective agent and (b) an intracellular portion of a T-cell receptor CD3, zeta or eta polypeptide, a B-cell receptor polypeptide or an Fc receptor polypeptide. Also claimed are: (1) DNA encoding a membrane-bound chimeric receptor comprising (a) an extracellular portion that specifically recognises and binds a target cell or a target infective agent and (b) a transmembrane portion of a T-cell receptor CD3, zeta or eta polypeptide, a B-cell receptor polypeptide or an Fc receptor polypeptide; (2) a vector containing the DNA of (I) or (1); (3) a membrane-bound chimeric receptor as in (I); (4) a membrane-bound chimeric receptor as in (1); (5) a membrane-bound chimeric receptor comprising (a) an extracellula r CD4 portion that specifically recognises and binds a target cell or a target infective agent and (b) an intracellular portion derived from a T-cell receptor, B-cell receptor or Fc receptor which is capable of signalling the cell to destroy a receptor-bound target cell or receptor-bound infective agent; (6) a membrane-bound chimeric receptor comprising (a) an extracellular CD4 portion that specifically recognises and binds a target cell or a target infective agent and (b) a

transmembrane portion derived from a T-cell receptor, B-cell receptor or Fc receptor which is capable of signalling the cell to destroy a receptor-bound target cell or receptor-bound infective agent; (7) a polypeptide consisting of T-cell receptor zeta amino acids 421-532, 423-455, 438-455, 461-494, 494-528 or 400-420 of a defined sequence of 532 amino acids (A) given in the specification; (8) cells expressing chimeric receptors as above.

USE - The cells can be administered to mammals in order to destroy pathogens (e.g. bacteria, fungi, protozoa or viruses, especially HIV), cancer cells or autoimmune-generated cells.

ABSTRACTED-PUB-NO: US 5843728A
EQUIVALENT-ABSTRACTS:

CHOSEN-DRAWING: Dwg.0/19

DERWENT-CLASS: B04 D16
CPI-CODES: B04-E02D; B04-E08; B04-F0100E; B04-K01; B04-N04A; B14-A01; B14-A02; B14-A02B1; B14-A03; B14-A04; B14-H01; D05-H12; D05-H12E; D05-H14; D05-H17A4;

L25 ANSWER 12 OF 22 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1993:641378 CAPLUS
DOCUMENT NUMBER: 119:241378
TITLE: Chimeric receptor genes, lymphocytes transformed with these genes, and use of the recombinant lymphocytes as tumor inhibitors
INVENTOR(S): Eshhar, Zelig; Schindler, Daniel; Waks, Tova; Gross, Gideon
PATENT ASSIGNEE(S): Yeda Research and Development Co., Ltd., Israel
SOURCE: PCT Int. Appl., 81 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
WO 9319163	A1	19930930	WO 1993-US2506	19930318	<--
W: AU, CA, JP, US					
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE					
AU 9339243	A1	19931021	AU 1993-39243	19930318	<--
AU 668156	B2	19960426			
EP 638119	A1	19950215	EP 1993-908414	19930318	<--
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE					
JP 07505282	T2	19950615	JP 1993-516731	19930318	<--
US 2002137697	A1	20020926	US 1995-547263	19951024	

PRIORITY APPLN. INFO.:

IL 1992-101288	A	19920318
IL 1993-104570	A	19930131
WO 1993-US2506	A	19930318
US 1993-84994	A2	19930702

AB Chimeric genes which contain a first segment encoding a single-chain Fv domain of a specific antibody and a second segment encoding at least the transmembrane and cytoplasmic domains of an immune cell-triggering mol. such as subunits of either a T cell receptor, a T cell receptor-CD3 complex, an Fc receptor, or an IL-2 receptor are described. A method of treatment of tumors comprises using lymphocyte cells transformed with expression vectors contg. the chimeric genes. Chimeric genes for anti-TNP antibody single-chain Fv fragment fused to **T cell receptor** .gamma. or .**zeta**. chain were prepd. MD.45 hybridoma cells transfected with these genes produced IL-2 upon exposure to TNP antigen, and lysed TNP-presenting target cells.

L25 ANSWER 2 OF 22 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1995:522862 CAPLUS
DOCUMENT NUMBER: 122:263540
TITLE: Genetically modified human hematopoietic stem cells and their progeny
INVENTOR(S): Mule, James
PATENT ASSIGNEE(S): Systemix Inc., USA
SOURCE: PCT Int. Appl., 38 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
WO 9506409	A1	19950309	WO 1994-US10033	19940906	<--
W: AU, CA, JP, US					
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE					
CA 2170980	AA	19950309	CA 1994-2170980	19940906	<--
AU 9477211	A1	19950322	AU 1994-77211	19940906	<--
AU 679120	B2	19970619			
EP 716570	A1	19960619	EP 1994-928018	19940906	
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE					
WO 9534676	A1	19951221	WO 1995-US3038	19950309	<--
W: AU, CA, JP, US, US					
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE					
AU 9521582	A1	19960105	AU 1995-21582	19950309	
EP 765398	A1	19970402	EP 1995-914698	19950309	
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE					
JP 10505483	T2	19980602	JP 1995-502123	19950309	
US 5985660	A	19991116	US 1995-482650	19950607	

PRIORITY APPLN. INFO.:

US 1993-116825	19930903
US 1994-260185	19940615
WO 1994-US10033	19940906
WO 1995-US3038	19950309
US 1995-464678	19950606

AB Human hematopoietic stem cells expressing recombinant DNA constructs encoding a recombinant mol. contg. a signal transducing region and an antigenic specificity region results in the generation of T cells with altered specificities. The signal transducing region is selected from T cell receptor, Fc receptor .gamma. chain of **TCR**, **TCR** . **zeta**., or IL-2 receptor .alpha., .beta., or .gamma. chain. The antigenic region is a antigen binding site derived from monoclonal antibody or VH and VL region of antibody specific for HIV-1 coat protein or tumor-assocd. surface antigen. The recombinant DNA may also contain a marker gene encoding G418 resistance or stable cell surface protein. In example, hematopoietic stem cells transduced with Sp6 vector carrying Fc receptor .gamma. chain and variable region of anti-TNP antibody were prepd. and analyzed. Hematopoietic stem cells carrying chimeric DNA encoding variable region of an IG recognizing HIV envelope gp160 or tumor-assocd. surface protein and .**zeta**. chain of **TCR** /CD3 complex or Fc receptor .gamma. were prepd. for cell therapy of HIV-1 infection or tumor resp.

L25 ANSWER 19 OF 22 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 1992-10807 BIOTECHDS
TITLE: New chimeric receptor protein activating secondary messenger pathways;
retro virus DNA cassette encoding CD3, CD4 or CD8 and Fc-epsilon-R1 receptor **fusion** protein, for expression on human cytotoxic T-lymphocyte cell surface for e.g. AIDS, cancer, hepatitis gene therapy
PATENT ASSIGNEE: Cell-Genesys
PATENT INFO: WO 9210591 **25 Jun 1992**
APPLICATION INFO: WO 1991-US9431 12 Dec 1991
PRIORITY INFO: US 1990-627643 14 Dec 1990
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1992-234646 [28]
AN 1992-10807 BIOTECHDS
AB A DNA sequence (I) encoding: a signal peptide sequence; an extracellular surface membrane protein domain (ED) (binding specifically to at least 1 ligand), optionally linked to a 2nd ED; a transmembrane domain (TD); and a cytoplasmic domain (CD) of the **TCR** associated **zeta** chain or eta chain, or the gamma chain of the Fc-epsilon-R1 receptor, is claimed. The ED and CD are not usually associated, while the TD and ED are. The ED is a heavy chain Ig in combination with a light chain or truncated light chain Ig, especially CD8 or CD4. The CD is especially the CD3 zeta chain. The following are new: (1) a DNA cassette with (I) and transcription control regions; (2) a human cytotoxic T-lymphocyte lacking surface expression of class-I or class-II major histocompatibility complex (MHC), expressing a surface membrane protein; (3) an ED protein; (4) an ED protein with CD3 zeta chain CD; (5) a method for activating cells via the secondary messenger pathway; and (6) a retro virus RNA or DNA cassette containing (I) for use in gene therapy of AIDS, hepatitis, cancer, etc. Activated T-lymphocytes may be used in therapy. (54pp)

L25 ANSWER 7 OF 22 MEDLINE

ACCESSION NUMBER: 96049639 MEDLINE

DOCUMENT NUMBER: 96049639 PubMed ID: 7486659

TITLE: Csk associates with the **TCR zeta** and epsilon chains through its SH2 domain.

AUTHOR: Rafnar T; Schneck J P; Brummet M E; Marsh D G; Catipovic B

CORPORATE SOURCE: Division of Clinical Immunology, Johns Hopkins Asthma and Allergy Center, Johns Hopkins University School of Medicine, Baltimore, Maryland 21224, USA.

SOURCE: ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, **(1995 Sep 7)** 766 206-8.
Journal code: 7506858. ISSN: 0077-8923.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199512

ENTRY DATE: Entered STN: 19960124
Last Updated on STN: 19960124
Entered Medline: 19951206

L25 ANSWER 11 OF 22 MEDLINE

ACCESSION NUMBER:	94053767 MEDLINE
DOCUMENT NUMBER:	94053767 PubMed ID: 7694368
TITLE:	Making molecular matches in the cell.
COMMENT:	Comment on: Science. 1993 Nov 12;262(5136):1019-24
AUTHOR:	Travis J
SOURCE:	SCIENCE, **(1993 Nov 12)** 262 (5136) 989.
	Journal code: 0404511. ISSN: 0036-8075.
PUB. COUNTRY:	United States
DOCUMENT TYPE:	Commentary
	News Announcement
LANGUAGE:	English
FILE SEGMENT:	Priority Journals
ENTRY MONTH:	199312
ENTRY DATE:	Entered STN: 19940117
	Last Updated on STN: 19970203
	Entered Medline: 19931217

L25 ANSWER 17 OF 22 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 93348490 MEDLINE
DOCUMENT NUMBER: 93348490 PubMed ID: 8346442
TITLE: Reconstitution of **T cell receptor zeta**-mediated calcium mobilization in nonlymphoid cells.
AUTHOR: Hall C G; Sancho J; Terhorst C
CORPORATE SOURCE: Division of Immunology, Beth Israel Hospital, Harvard Medical School, Boston, MA 02115.
CONTRACT NUMBER: AI 15066 (NIAID)
CA 01486 (NCI)
SOURCE: SCIENCE, **(1993 Aug 13)** 261 (5123) 915-8.
Journal code: 0404511. ISSN: 0036-8075.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199309
ENTRY DATE: Entered STN: 19930924
Last Updated on STN: 19980206
Entered Medline: 19930908

AB T cell antigen receptor (TCR) activation involves interactions between receptor subunits and nonreceptor protein tyrosine kinases (PTKs). Early steps in signaling through the zeta chain of the TCR were examined in transfected COS-1 cells. Coexpression of the PTK p59fynT, but not p56lck, with zeta or with a homodimeric **TCR** beta-**zeta fusion** protein produced tyrosine phosphorylation of both zeta and phospholipase C (PLC)-gamma 1, as well as calcium ion mobilization in response to receptor cross-linking. CD45 coexpression enhanced these effects. No requirement for the PTKZAP-70 was observed. Thus, p59fynT may link zeta directly to the PLC-gamma 1 activation pathway.

L25 ANSWER 15 OF 22 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 94065576 MEDLINE
DOCUMENT NUMBER: 94065576 PubMed ID: 7504057
TITLE: **T cell receptor zeta** /CD3-p59fyn(T)-associated p120/130 binds to the SH2 domain of p59fyn(T).
AUTHOR: da Silva A J; Janssen O; Rudd C E
CORPORATE SOURCE: Division of Tumor Immunology, Dana-Farber Cancer Institute, Boston, Massachusetts.
CONTRACT NUMBER: RO1 12069
SOURCE: JOURNAL OF EXPERIMENTAL MEDICINE, **(1993 Dec 1)** 178 (6) 2107-13.
Journal code: 2985109R. ISSN: 0022-1007.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199312
ENTRY DATE: Entered STN: 19940201
Last Updated on STN: 20000303
Entered Medline: 19931228

AB Intracellular signaling from the **T cell receptor** (**TCR**)**zeta**/CD3 complex is likely to be mediated by associated protein tyrosine kinases such as p59fyn(T), ZAP-70, and the CD4:p56lck and CD8:p56lck coreceptors. The nature of the signaling cascade initiated by these kinases, their specificities, and downstream targets remain to be elucidated. The **TCR-zeta**/CD3:p59fyn(T) complex has previously been noted to coprecipitate a 120/130-kD doublet (p120/130). This intracellular protein of unknown identity associates directly with p59fyn(T) within the receptor complex. In this study, we have shown that this interaction with p120/130 is specifically mediated by the SH2 domain (not the fyn-SH3 domain) of p59fyn(T). Further, based on the results of in vitro kinase assays, p120/130 appears to be preferentially associated with p59fyn(T) in T cells, and not with p56lck. Antibody reprecipitation studies identified p120/130 as a previously described 130-kD substrate of pp60v-src whose function and structure is unknown. **TCR-zeta**/CD3 induced activation of T cells augmented the tyrosine phosphorylation of p120/130 in vivo as detected by antibody and GST:fyn-SH2 **fusion** proteins. p120/130 represents the first identified p59fyn(T):SH2 binding substrate in T cells, and as such is likely to play a key role in the early events of T cell activation.

L17 ANSWER 4 OF 4 MEDLINE DUPLICATE 1

ACCESSION NUMBER: 91139175 MEDLINE
DOCUMENT NUMBER: 91139175 PubMed ID: 1671667
TITLE: Haplotypic polymorphisms of the **TNFB gene**

AUTHOR: Abraham L J; Du D C; Zahedi K; Dawkins R L; Whitehead A S
CORPORATE SOURCE: Department of Clinical Immunology, Royal Perth Hospital, Western Australia.

SOURCE: IMMUNOGENETICS, **(1991)** 33 (1) 50-3.
Journal code: 0420404. ISSN: 0093-7711.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M55913; GENBANK-M74709; GENBANK-M76774; GENBANK-M76775; GENBANK-M76776; GENBANK-M76777; GENBANK-M76778; GENBANK-M76779; GENBANK-M76780; GENBANK-S40906
ENTRY MONTH: 199103
ENTRY DATE: Entered STN: 19910412
Last Updated on STN: 19950206
Entered Medline: 19910327

AB The **TNFB genes** from two major histocompatibility complex (MHC) ancestral haplotypes have been compared. The genes carried by the ancestral haplotypes 8.1 (A1,B8,BfS,C4AQ0, C4B1,DR3) and 57.1 (A1, B57. BfS, C4A6, C4B1, DR7) were cloned and sequenced to determine the degree of polymorphism. In this report we show that the respective TNF genes are allelic and have unique nucleotide sequences. The data demonstrate the presence of three nucleotide differences between the TNFB alleles of 8.1 and 57.1. Two of the differences occur in untranslated regions of the **gene** but the third nucleotide change results in an amino acid difference in the mature **TNFB** protein. These polymorphisms may have implications with respect to differential regulation in disease- and nondisease-associated haplotypes.

L20 ANSWER 13 OF 13 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 1994-14073 BIOTECHDS
TITLE: Design of interchain disulfide bonds in the framework of Fv fragments;
disulfide bond introduction by protein engineering; single
chain antibody engineering and immunotoxin production (conference abstract)
AUTHOR: Jung S H; Pastan I; Lee B K
CORPORATE SOURCE: Nat.Cancer-Inst.Bethesda; Nat.Inst.Health-Bethesda
LOCATION: Laboratory of Molecular Biology, National Cancer Institute, NIH, Bethesda, MD 20892, USA.
SOURCE: J.Cell.Biochem.; (1994) Suppl.18D, 207
CODEN: JCEBD5
DOCUMENT TYPE: Journal
LANGUAGE: English
AN 1994-14073 BIOTECHDS
AB Molecular modeling tools were used to identify possible interchain disulfide bond sites in the framework (FR) region of the Fv model structure of the mouse monoclonal antibody (MAb) B3. 2 Sites were identified (VH44-VL105 and VH111-VL48), which were related by a 2-fold pseudorotational symmetry. Selection criteria and the modeling scheme were described. Since these disulfide bond sites were in the FR region, they could be located for the Fv of any Ig molecule from sequence alignment alone. To test this design, interchain disulfide bonds were introduced by site-directed mutagenesis in the Fv fragments of 3 different antibodies, MAb B3, **e23** and anti-Tac, each fused to a truncated form of Pseudomonas sp. exotoxin. All 3 immunotoxins were just
as active as the corresponding **single chain** antibody counterparts, and considerably more stable. The purification yield was also significantly higher. (5 ref)

L2 ANSWER 1 OF 1 PCTFULL COPYRIGHT 2003 Univentio
ACCESSION NUMBER: 1995030014 PCTFULL ED 20020514
TITLE (ENGLISH): BIFUNCTIONAL PROTEIN, PREPARATION AND USE
TITLE (FRENCH): PROTEINE A DOUBLE FONCTION, SA PREPARATION ET SON UTILISATION
INVENTOR(S): **GRONER, Bernd;**
MORITZ, Dirk
PATENT ASSIGNEE(S): CIBA-GEIGY AG;
GRONER, Bernd;
MORITZ, Dirk
LANGUAGE OF PUBL.: German
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9530014	A1	19951109

DESIGNATED STATES
W: AM AU BB BG BR BY CA CN CZ EE FI GE HU IS JP KG KP KR KZ LK LR LT LV MD MG MN MX NO NZ PL RO RU SG SI SK TJ TM TT UA US UZ VN KE MW SD SZ UG AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG

APPLICATION INFO.: WO 1995-EP1494 A 19950420
PRIORITY INFO.: AT 1994-94810244.7 19940502

L1 ANSWER 15 OF 17 PCTFULL COPYRIGHT 2003 Univentio

ACCESSION NUMBER: 1995030014 PCTFULL ED 20020514

TITLE (ENGLISH): BIFUNCTIONAL PROTEIN, PREPARATION AND USE

TITLE (FRENCH): PROTEINE A DOUBLE FONCTION, SA PREPARATION ET SON UTILISATION

INVENTOR(S): **GRONER, Bernd;**
MORITZ, Dirk

PATENT ASSIGNEE(S): CIBA-GEIGY AG;
GRONER, Bernd;
MORITZ, Dirk

LANGUAGE OF PUBL.: German

DOCUMENT TYPE: Patent

PATENT INFORMATION:

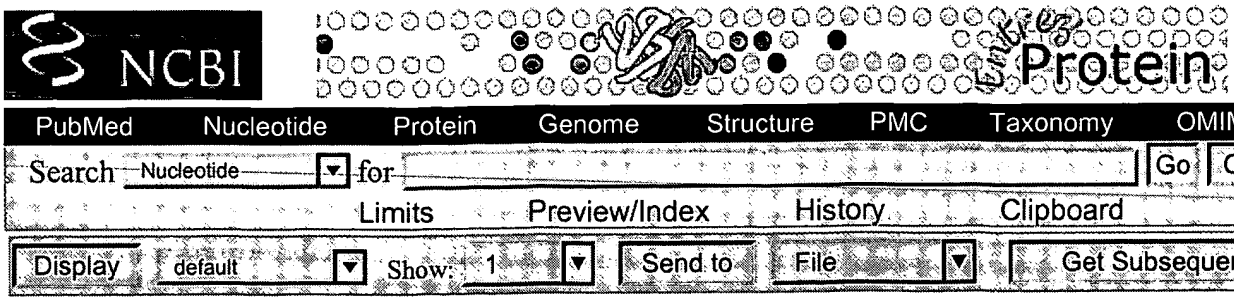
NUMBER	KIND	DATE
WO 9530014	A1	19951109

DESIGNATED STATES
W: AM AU BB BG BR BY CA CN CZ EE FI GE HU IS JP KG KP KR KZ LK LR LT LV MD MG MN MX NO NZ PL RO RU SG SI SK TJ TM TT UA US UZ VN KE MW SD SZ UG AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG

APPLICATION INFO.: WO 1995-EP1494 A 19950420

PRIORITY INFO.: AT 1994-94810244.7 19940502

L25 ANSWER 3 OF 5 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 1994-14077 BIOTECHDS
TITLE: A diabody for imaging of atherosclerotic plaques; monoclonal antibody single chain antibody Fv fragment diabody production for potential atherosclerosis plaque imaging, restenosis diagnosis, etc. (conference abstract)
AUTHOR: Adair J; Armour K; Carr F; Ditlow C; Chen F; Holliger P
CORPORATE SOURCE: Scotgen-Biopharm.; Med.Res.Counc.
LOCATION: Scotgen Biopharmaceuticals Inc., Research Centre, Kettock Lodge, Aberdeen Science and Technology Park, Bridge of Don, Aberdeen, AB22 8GU, Scotland, UK.
SOURCE: J.Cell.Biochem.; (**1994**) Suppl.18D, 209
CODEN: JCEBD5
DOCUMENT TYPE: Journal
LANGUAGE: English
AN 1994-14077 BIOTECHDS
AB Monoclonal antibody Z2D3 (IgM/kappa) recognizes a novel antigen presented on atherosclerotic plaques (APs) and has potential for imaging and therapy of atherosclerosis. For imaging, a molecule of low mol.wt. would be preferred because of its rapid clearance from the rest of the body and its penetration to the antigen throughout the APs. Monomeric antibody fragments (Fab, Fv or single chain Fv (**scFv**)) usually lead to lower binding avidity compared to **bivalent** F(ab')2 or IgG. A **bivalent** low mol.wt. alternative was sought. When Fv with short (less than 10 amino acids) peptide linkers was expressed in bacteria, a proportion of the **scFv** spontaneously dimerized and formed a diabody, which had antigen binding activity similar to that of whole IgG. Diabodies were the main product when the peptide linker was removed and the 2 domains of the **scFv** were directly linked. A diabody was constructed using the variable regions from Z2D3. The diabody had binding **affinity** for APs antigens similar to that of a Z2D3 mouse-human chimeric antibody and a chimeric F(ab')2 fragment. The diabody was used for radio imaging and for restenosis, etc., diagnosis. (0 ref)



☐ 1: AAA60394. T-cell receptor z...[gi:623042] BLink, Links

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LOCUS       AAA60394                163 aa            linear   PRI 13-JAN-1995
DEFINITION  T-cell receptor zeta chain.
ACCESSION   AAA60394
VERSION     AAA60394.1  GI:623042
DBSOURCE    locus HUMTCRZCN accession J04132.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1  (residues 1 to 163)
  AUTHORS   Weissman,A.M., Hou,D., Orloff,D.G., Modi,W.S., Seuanez,H.,
            O'Brien,S.J. and Klausner,R.D.
  TITLE     Molecular cloning and chromosomal localization of the human T-cell
            receptor zeta chain: distinction from the molecular CD3 complex
  JOURNAL   Proc. Natl. Acad. Sci. U.S.A. 85 (24), 9709-9713 (1988)
  MEDLINE   89071765
   PUBMED   2974162
COMMENT     Method: conceptual translation.
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L6 ANSWER 19 OF 22 MEDLINE DUPLICATE 17
ACCESSION NUMBER: 92308829 MEDLINE
DOCUMENT NUMBER: 92308829 PubMed ID: 1351920
TITLE: The CD3 zeta cytoplasmic domain mediates CD2-induced T cell activation.
AUTHOR: Howard F D; Moingeon P; Moebius U; McConkey D J; Yandava B; Gennert T E; Reinherz E L
CORPORATE SOURCE: Laboratory of Immunobiology, Dana-Farber Cancer Institute, Boston, Massachusetts.
CONTRACT NUMBER: AI-19807 (NIAID)
SOURCE: JOURNAL OF EXPERIMENTAL MEDICINE, **(1992 Jul 1)** 176 (1) 139-45.
Journal code: I2V; 2985109R. ISSN: 0022-1007.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199207
ENTRY DATE: Entered STN: 19920807
Last Updated on STN: 19950206
Entered Medline: 19920729

AB CD2-mediated T lymphocyte activation requires surface expression of CD3-Ti, the **T cell receptor** (**TCR**) for antigen major histocompatibility complex protein. Given the importance of CD3 **zeta** in **TCR** signaling, we have directly examined the ability of the CD3 **zeta** cytoplasmic domain to couple CD2 to intracellular signal transduction pathways. A cDNA encoding a **chimeric** protein consisting of the human CD3 **zeta** cytoplasmic domain (amino acid residues 31-142) fused to the CD8 alpha extracellular and transmembrane domains (amino acid residues 1-187) was transfected into a CD2+CD3-CD8- variant of the human T cell line Jurkat. The resulting transfectants expressed the CD8 alpha/CD3 **zeta** **chimeric** receptor at the cell surface in the absence of other **TCR** subunits. Stimulation of these transfectants with anti-T11(2) + anti-T11(3) monoclonal **antibodies** (mAbs) initiated both a prompt cytosolic free calcium ([Ca2+]i) rise and protein tyrosine kinase activation. Stimulation with either intact anti-T11(2) + anti-T11(3) mAbs or purified F(ab')2 fragments resulted in interleukin 2 (IL-2) secretion. In contrast, control cell lines transfected with a cDNA encoding wild-type CD8 alpha, and thus lacking surface expression of the CD3 **zeta** cytoplasmic domain, failed to show any [Ca2+]i rise, protein tyrosine kinase activation, or IL-2 secretion after identical stimulation. These data directly establish the CD3 **zeta** cytoplasmic domain as a necessary and sufficient component of the CD3-Ti complex involved in T lymphocyte activation through CD2. Moreover, they show that CD2 signaling can function in the absence of Fc receptors.

L6 ANSWER 3 OF 22 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 95236300 MEDLINE
DOCUMENT NUMBER: 95236300 PubMed ID: 7719942
TITLE: CD3 epsilon and CD3 zeta cytoplasmic domains can independently generate signals for T cell development and function.
AUTHOR: Shinkai Y; Ma A; Cheng H L; Alt F W
CORPORATE SOURCE: Howard Hughes Medical Institute, Department of Genetics, Children's Hospital, Boston, Massachusetts, USA.
CONTRACT NUMBER: A120047 (NCI)
CA42335
U01 A131541
SOURCE: IMMUNITY, **(1995 Apr)** 2 (4) 401-11.
Journal code: CCF; 9432918. ISSN: 1074-7613.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199505
ENTRY DATE: Entered STN: 19950605
Last Updated on STN: 19970203
Entered Medline: 19950525

AB To determine whether CD3 epsilon and CD3 **zeta** proteins have unique roles in **TCR**-dependent functions, **chimeric** genes encoding the extracellular and transmembrane domains of the human IL-2 receptor alpha chain (Tac) fused to a cytoplasmic domain of either the CD3 epsilon or CD3 **zeta** chain were introduced as transgenes into both normal and RAG2-deficient (RAG2-/-) mice. Developmental arrest of T lineage cells at the CD4, CD8 double-negative stage in the transgenic RAG2-/- thymus was released to the CD4, CD8 double-positive (DP) stage by in vivo cross-linking of TT epsilon or TT **zeta** with anti-Tac **antibody**. In TT epsilon + or TT **zeta** +, RAG2-/- mice, in vitro cross-linking of TT epsilon and TT **zeta** induced DP thymocyte cell death and proliferation of mature single-positive T cells. Overall, no qualitative differences were observed between TT epsilon- and TT **zeta**-mediated functions, suggesting that different CD3 components deliver qualitatively similar signals in inducing **TCR** -dependent functions.

L6 ANSWER 4 OF 22 BIOSIS

L6 ANSWER 4 OF 22 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1995:534030 BIOSIS
DOCUMENT NUMBER: PREV199598548330
TITLE: T-bodies: chimeric T-cell receptors with antibody-type specificity.
AUTHOR(S): Eshhar, Zelig (1); Gross, Gideon; Waks, Tova; Lustgarten, Joseph; Bach, Nathan; Ratner, Anna; Treisman, Jonathan; Schindler, Daniel G.
CORPORATE SOURCE: (1) Dep. Chem. Immunol., Weizmann Inst. Sci., Rehovot 76100
Israel
SOURCE: Methods (Orlando), (1995) Vol. 8, No. 2, pp. 133-142. ISSN: 1046-2023.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The variable regions of the **T-cell receptor** for antigen (**TCR**) and **antibodies** serve as recognition elements, enabling T and B cells to bind antigen with high specificity. Nevertheless, the complex recognition requirements of T cells, which respond to antigen only in the context of a self MHC molecule, do not permit the generation of antigen-specific lymphocytes, which could serve as universal effector cells. To enable the generation of non-MHC-restricted T cells of predetermined specificity, we took advantage of the similarity between the **antibody** and the T-cell molecule and expressed in T-cell lines **chimeric T-cell receptor** (cTCR) genes in which the **TCR** variable region is replaced by an **antibody** variable region of defined specificity. In another design, the **antibody** recognition unit has been used in the form of a single-chain Fv (scFv) to create hybrid molecules with other receptor subunits, including the **zeta** chain of the CD3 complex and the gamma chain of the high-affinity receptor for IgE (Fc-epsilon-RI). T-cell lines transfected with the **chimeric TCR** or scFvR exhibit cytotoxicity or secrete cytokines in response to antigen displayed either on a solid substrate or on the surface of target cells. We describe here the various configurations of the **chimeric** receptors that we have produced. Technical aspects, including the choice of vector, **antibody** sequences, and triggering molecule, are reviewed.

L6 ANSWER 5 OF 22 MEDLINE DUPLICATE 4

L6 ANSWER 6 OF 22 CANCERLIT
ACCESSION NUMBER: 96606995 CANCERLIT
DOCUMENT NUMBER: 96606995
TITLE: Cancer targeting using genetically engineered effector lymphocytes (Meeting abstract).
AUTHOR: Eshhar Z; Waks T; Gross G; Lustgarten J; Bach N; Fitzer-Attas C J; Ratner A; Schindler D G
CORPORATE SOURCE: Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel.
SOURCE: Gene Ther, **(1995)**. Vol. 2, Suppl. 1:S20.
ISSN: 0969-7128.
DOCUMENT TYPE: (MEETING ABSTRACTS)
FILE SEGMENT: ICDB
LANGUAGE: English
ENTRY MONTH: 199605

AB Tumor-specific T cells are very rare and can be derived and applied for adoptive immunotherapy only in a limited number of malignancies. Monoclonal **antibodies**, on the other hand, can be made quite readily against many tumor associated antigens, however, their therapeutic application is limited; they can be effective in the elimination of blood borne tumor cells yet they do not efficiently penetrate solid tumors. In order to redirect effector lymphocytes to predefined targets in non-MHC restricted manner, we have used **chimeric** receptor genes consisting of **antibody** variable regions combined with cell activating domains. Several basic designs have been constructed. The first was composed of the two **chimeric T cell receptor** chains (cTCR) in which the constant regions of the **T cell receptor** chains were combined with the VH and VL of specific monoclonal **antibody**. The second design employed a single-chain Fv of **antibody** as the recognition unit, linked to the signal transducing gamma or **zeta** subunits of the FcR or CD3 (scFvR) in one continuous polypeptide chain. In the scfvr, the spacing of the scfv from the transmembrane moiety using flexible hinge domains has been found helpful. Practically, the use of the scfvr design enables the use of more efficient gene delivery systems such as retrovectors. Furthermore, it allows the use of several lymphocyte triggering receptor subunits, thus extending the application of the **chimeric** receptor (T-Body) approach to different cell types, including lymphoid and nonlymphoid ones. In a third design, we bypassed the **TCR** complex and connected the extracellular **antibody** recognition unit through CD4 or CD8 transmembranal stretch directly to intracellular protein-tyrosine kinases (PTK). Following transfection or transduction into T cells, the **chimeric** genes were expressed as functional surface receptors and conferred non-MHC restricted, **antibody**-type specificity on the recipient cells. Upon encountering antigen (either immobilized or displayed on target cells), the **chimeric** receptors triggered T cell activation. Soluble, mono- or multivalent antigens did not stimulate and rather inhibited activation of T cells. Syk was found to be better than ZAP-70 as the intracellular kinase in the scfv-PTK **chimeric** receptor design. It could transmit signals leading to phosphorylation, IL-2 release and specific target cell cytolysis. When Fv of antihuman carcinoma **antibodies** such as the anti-HER2 or antifolate binding protein **antibodies** were employed, cytotoxic lymphocytes (either CTL or NK) harboring the **chimeric** genes could specifically lyse the corresponding human tumor target cells. Such 'T-Body' approach holds promise for gene immunotherapy of cancer by directing effector lymphocytes

(either as such, or harboring any transgene of interest) to the tumor site.

L6 ANSWER 7 OF 22 MEDLINE DUPLICATE 5

L6 ANSWER 8 OF 22 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 95108016 MEDLINE
DOCUMENT NUMBER: 95108016 PubMed ID: 7809095
TITLE: Functional three-domain single-chain T-cell receptors.
AUTHOR: Chung S; Wucherpfennig K W; Friedman S M; Hafler D A; Strominger J L
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138.
CONTRACT NUMBER: CA47554 (NCI)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, **(1994 Dec 20)** 91 (26) 12654-8.
Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199502
ENTRY DATE: Entered STN: 19950215
Last Updated on STN: 19970203
Entered Medline: 19950202

AB **T-cell receptors** (**TCRs**) are membrane anchored heterodimers structurally related to **antibody** molecules. Single-chain **antibodies** can be **engineered** by linking the two variable domains, which fold properly by themselves. However, proper assembly of the variable domains of a human **TCR** (V alpha and V beta) that recognize the HLA-DR2b/myelin basic protein-(85-99) peptide complex was critically dependent on the addition of a third domain, the constant region of the **TCR** beta chain (C beta), to the single-chain construct. Single-chain molecules with the three-domain design, but not those with the two-domain design, expressed in a eukaryotic cell as **chimeric** molecules linked either to glycosyl phosphatidylinositol or to the transmembrane/cytoplasmic domains of the CD3 **zeta** chain were recognized by a conformation-sensitive monoclonal **antibody**. The **chimeric** three-domain single-chain **TCR** linked to CD3 **zeta** chain signaled in response to both the specific HLA-DR/peptide and the HLA-DR/superantigen staphylococcal enterotoxin B complexes. Thus, by using this three-domain design, functional single-chain **TCR** molecules were expressed with high efficiency. The lipid-linked single-chain **TCR** was solubilized by enzymatic cleavage and purified by affinity chromatography. The apparent requirement of the constant domain for cooperative folding of the two **TCR** variable domains may reflect significant structural differences between **TCR** and **antibody** molecules.

L6 ANSWER 9 OF 22 MEDLINE DUPLICATE 7

L6 ANSWER 9 OF 22 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 94240128 MEDLINE
DOCUMENT NUMBER: 94240128 PubMed ID: 7910405
TITLE: Cytotoxic T lymphocytes with a grafted recognition specificity for ERBB2-expressing tumor cells.
AUTHOR: Moritz D; Wels W; Mattern J; Groner B
CORPORATE SOURCE: Friedrich Miescher-Institute, Basel, Switzerland.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, **(1994 May 10)** 91 (10) 4318-22.
Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199406
ENTRY DATE: Entered STN: 19940621
Last Updated on STN: 20000303
Entered Medline: 19940616

AB Experimental approaches which exploit the targeted cytolytic activity of lymphocytes are being developed for cancer therapy. We generated cytotoxic T lymphocytes (CTLs) with specificity for ERBB2 receptor-expressing tumor cells. A binding function was conferred directly on the **zeta** chain of the **T-cell receptor** (**TCR**) complex to circumvent major histocompatibility complex-restricted antigen recognition through the alpha and beta chains of the **TCR**. A **chimeric** gene was constructed which encoded a single-chain Fv **antibody** (scFv, consisting of the joined heavy- and light-chain variable domains of a monoclonal **antibody** against the extracellular domain of the ERBB2 receptor), a hinge region as a spacer, and the **zeta** chain of the **TCR**. This gene was introduced into CTLs by retroviral gene transfer. The signaling potential of the scFv/hinge/**zeta** receptors was demonstrated by secretion of interferon gamma upon coincubation with ERBB2-expressing cells. Target cells expressing the ERBB2 gene were lysed in vitro with high specificity by the scFv/hinge/**zeta**-expressing T cells. The growth of ERBB2-transformed cells in athymic nude mice was retarded by adoptively transferred scFv/hinge/**zeta**-expressing CTLs. Transduced CTLs labeled with a fluorescent dye were specifically detected in tumor sections. Our results suggest that tumor cell lysis by CTLs grafted in vitro with a major histocompatibility complex-independent recognition could become a gene-therapy approach to cancer treatment.

L6 ANSWER 10 OF 22 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 94194272 MEDLINE

lymphocytes.

L6 ANSWER 12 OF 22 MEDLINE DUPLICATE 10

L6 ANSWER 11 OF 22 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 94194254 MEDLINE
DOCUMENT NUMBER: 94194254 PubMed ID: 8145022
TITLE: Use of the 5'-flanking region of the mouse perforin gene to express human Fc gamma receptor I in cytotoxic T lymphocytes.
AUTHOR: Smyth M J; Kershaw M H; Hulett M D; McKenzie I F; Trapani J A
CORPORATE SOURCE: Cellular Cytotoxicity Laboratory, Austin Research Institute, Austin Hospital, Heidelberg, Victoria, Australia.
SOURCE: JOURNAL OF LEUKOCYTE BIOLOGY, **(1994 Apr)** 55 (4) 514-22.
Journal code: IWY; 8405628. ISSN: 0741-5400.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199405
ENTRY DATE: Entered STN: 19940511
Last Updated on STN: 19940511
Entered Medline: 19940505

AB Expression of the gene encoding the cytolytic granule protein perforin is restricted to cytotoxic lymphocytes. To undertake a functional analysis of the immediate 5'-promoter region of the mouse perforin gene, we transiently transfected mouse perforin promoter-chloramphenicol acetyltransferase (CAT) reporter gene constructs into cytotoxic T, T lymphoid, B-lymphoid, and nonlymphoid cell lines. The transcriptional activity of the perforin promoter was restricted to cytotoxic lymphocytes.

The perforin promoter was controlled by several positive (in perforin-positive cells) and negative (in perforin-negative cells) cis-acting regions, spread over at least 1.1 kilobases. The most specific expression of the CAT reporter gene in the interleukin-2-dependent cytotoxic T cell line CTLL-R8 was obtained with the mouse perforin promoter encompassing positions -1104 to +1 in relation to the RNA cap site. This construct expressed 65- to 70-fold higher CAT activity than the promoterless CAT construct in perforin-expressing cells but only 1- to 5-fold higher CAT activity than the promoterless construct in nonlymphoid cells. On the basis of these data, we used this most specifically active mouse perforin promoter, -1104 to +1, to express in CTLL-R8, a **chimeric** human receptor comprising the extracellular domains of human Fc gamma RI and the transmembrane and intracellular domains of **TCR zeta**. Selection in G418-containing medium produced CTLL-R8 transfectant clones that (1) expressed high levels of human Fc gamma RI mRNA; (2) expressed cell surface Fc gamma RI as demonstrated by immunoprecipitation and their ability to bind the Fc portion of human and mouse monoclonal **antibodies** (mAbs) in an isotype-specific manner, and (3) bound RBC expressing mucin-1 (Muc-1) peptide in the presence of a **chimeric** mouse-human anti-Muc-1 mAb. Activation of CTLL-R8 transfectants upon engagement of the human Fc gamma RI was evidenced by their ability to lyse tumor target cells in an mAb isotype-dependent manner. The successful expression of a functional **chimeric** gene in CTLL-R8 suggests that the mouse perforin promoter represents a novel reagent for expressing exogenous genes in cytotoxic T

L6 ANSWER 18 OF 22 MEDLINE DUPLICATE 16
ACCESSION NUMBER: 94080048 MEDLINE
DOCUMENT NUMBER: 94080048 PubMed ID: 8257803
TITLE: Branched polypeptides as antigens for influenza virus hemagglutinin and T-cell receptor subunits.
AUTHOR: Toth G K; Varadi G; Nagy Z; Monostori E; Penke B; Hegedus Z; Ando I; Fazekas G; Kurucz I; Mak M; +
CORPORATE SOURCE: A. Szent-Gyorgyi Medical University, Szeged, Hungary.
SOURCE: PEPTIDE RESEARCH, **(1993 Sep-Oct)** 6 (5) 272-80.
Journal code: BE1; 8913494. ISSN: 1040-5704.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199401
ENTRY DATE: Entered STN: 19940203
Last Updated on STN: 19970203
Entered Medline: 19940119

AB The multiple antigenic peptide (MAP) method was applied to improve the immunogenicity of synthetic peptides representing distinct regions of the influenza virus hemagglutinin (HA). A tetrameric MAP with multiply incorporated overlapping B- and T-cell epitopes was combined with a particular HA sequence representing the slightly modified **fusion** peptide on the C-terminus of the Lys core (MAP-1). As a result of repeated injections of BALB/c mice with MAP-1 but not with the monomeric HA1C[Arg] peptide, the appearance of MAP-1-specific **antibodies** crossreactive with the acid-pretreated virus could be observed. In vitro studies revealed the potency of the MAP-1 structure to induce proliferation of HA1C[Arg]-primed T-cells, and in vivo studies demonstrated the protective feature of the immune response elicited by MAP-1 and to a lesser extent by the monomeric HA1C[Arg]. The increased level of MAP-1 specific **antibodies** upon viral challenge shows the activation of MAP-1-specific B- and/or T-cells. The advantage of the previously verified FP3 helper T-cell epitope included in MAP-1 was further utilized to synthesize **chimeric** structures comprising short fragments of the **zeta** of (MAP-2) or delta (MAP-3) subunits of the **T-cell** antigen **receptor** (**TCR**) complex. The selected peptides of the **zeta** and delta-chain regions failed to elicit an **antibody** response in BALB/c mice as tetra- or octamers, but the inclusion of the modified **fusion** region resulted in an immunogenic construction. (ABSTRACT TRUNCATED AT 250 WORDS)

L6 ANSWER 19 OF 22 MEDLINE DUPLICATE 17

L25 ANSWER 6 OF 14 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 94143355 MEDLINE
DOCUMENT NUMBER: 94143355 PubMed ID: 8309948
TITLE: An improved linker for single-chain Fv with reduced aggregation and enhanced proteolytic stability.
AUTHOR: Whitlow M; Bell B A; Feng S L; Filpula D; Hardman K D; Hubert S L; Rollence M L; Wood J F; Schott M E; Milenic D E; +
CORPORATE SOURCE: Protein Engineering Department, Enzon, Incorporated, Piscataway, NJ 08854-3998.
SOURCE: PROTEIN ENGINEERING, **(1993 Nov)** 6 (8) 989-95.
Journal code: PR1; 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199403
ENTRY DATE: Entered STN: 19940330
Last Updated on STN: 19940330
Entered Medline: 19940316

AB The effects of linker length on binding affinity and degree of aggregation have been examined in the antifluorescein 4-4-20 and anticarcinoma CC49 single-chain Fvs. Longer **linkers** in the antifluorescein **sFvs** have higher affinities for fluorescein and aggregate less. A proteolytically susceptible site between Lys8 and Ser9, in the previously reported 212 linker has been identified. A new linker sequence, 218 (GSTSGSGKPGSGEGSTKG) was designed in which a proline was placed at the C-terminal side of the proteolytic clip site in the 212 linker. The CC49 **sFv** containing the 218 linker showed reduced aggregation and was found to be more stable to proteolysis in vitro, when compared to the CC49/212 **sFv**. The CC49 **sFv** with the longer 218 linker had higher affinity than CC49/212 **sFv**. An aggregated CC49/212 **sFv** sample had higher affinity than CC49/218 **sFv**. The CC49/218 and CC49/212 **sFvs** had similar blood clearances in mice, while the aggregated CC49/212 **sFv** remained in circulation significantly longer. In mice bearing LS-174T human colon carcinoma xenografts, the CC49/218 **sFv** showed higher tumor uptake than the CC49/212 **sFv** and lower tumor uptake than the aggregated CC49/212 **sFv**. The higher tumor uptake of the CC49/218 is most likely a result of its higher resistance to proteolysis. The higher affinity and higher tumor uptake of the aggregated CC49/212 **sFv** are most likely due to the repetitive nature of the TAG-72 antigen and the higher avidity of multivalent aggregates. When the **sFvs** were radiolabeled with a lutetium-chelate the CC49/218 **sFv** showed a lower accumulation in the liver and spleen compared to the aggregated CC49/212 **sFv**.

L6 ANSWER 2 OF 22 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 96115535 MEDLINE
DOCUMENT NUMBER: 96115535 PubMed ID: 8593604
TITLE: A spacer region between the single chain **antibody** - and the CD3 **zeta**-chain domain of **chimeric T cell receptor** components is required for efficient ligand binding and signaling activity.
AUTHOR: Moritz D; Groner B
CORPORATE SOURCE: Institute for Experimental Cancer Research, Tumor Biology Center, Freiburg, Germany.
SOURCE: GENE THERAPY, **(1995 Oct)** 2 (8) 539-46.
Journal code: CCE; 9421525. ISSN: 0969-7128.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199604
ENTRY DATE: Entered STN: 19960422
Last Updated on STN: 19970203
Entered Medline: 19960410

AB The elimination of tumor cells by cytotoxic T lymphocytes (CTLs) could become the basis of a biological cancer therapy. The recognition specificity of cytotoxic T lymphocytes (CTLs) can be genetically modified by stable introduction of **chimeric T cell receptor** genes and thus be directed towards tumor cells. We designed a **recombinant T cell receptor** (**TCR**) component consisting of a single chain Fv derivative of a monoclonal **antibody** (scFv) serving as the extracellular antigen-binding domain and the **zeta**-chain of the **TCR**/CD3 complex serving as a signal transducing domain. Three **chimeric** receptor constructs differing in their molecular structure were derived and their functions in transduced T cells compared.

A construct in which the scFv domain, specific for the ErbB-2 receptor, was fused directly to the **zeta**-chain, and two constructs containing different hinge regions between the functional domains, were made. The hinge regions serve as spacers which increase the distance of the scFv moiety from the plasma membrane. Only the two scFv-**zeta chimeras** containing a hinge region showed ErbB-2 binding activity, when expressed in T cells. The scFv-**zeta** construct which lacks a spacer segment did not. Consistently, only the spacer-containing **chimeras** transduced **T cell receptor** signals following ErbB-2 mediated crosslinking. An increase in intracellular Ca2+ concentrations and cytokine secretion was observed. ErbB-2 expressing tumor cells were efficiently lysed by CTLs which expressed the spacer-containing scFv-**zeta chimeras**. Our results will help to optimize the design of **recombinant T cell receptor** components useful in the grafting of a specificity of recognition on to cytotoxic T cells and possibly the gene therapy of cancer.

L6 ANSWER 3 OF 22 MEDLINE DUPLICATE 3

L20 ANSWER 12 OF 17 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 93364902 MEDLINE
DOCUMENT NUMBER: 93364902 PubMed ID: 7689421
TITLE: Highly specific in vivo tumor targeting by monovalent and divalent forms of 741F8 anti-c-erbB-2 single-chain Fv.
AUTHOR: Adams G P; McCartney J E; Tai M S; Oppermann H; Huston J S;
Stafford W F 3rd; Bookman M A; Fand I; Houston L L; Weiner L M
CORPORATE SOURCE: Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111.
CONTRACT NUMBER: CA06927 (NCI)
U01 CA51880 (NCI)
SOURCE: CANCER RESEARCH, **(1993 Sep 1)** 53 (17) 4026-34.
Journal code: CNF; 2984705R. ISSN: 0008-5472.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
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ENTRY MONTH: 199309
ENTRY DATE: Entered STN: 19931015
Last Updated on STN: 20000303
Entered Medline: 19930924

AB The in vivo properties of monovalent and divalent single-chain Fv (**sFv**)-based molecules with the specificity of the anti-c-erbB-2 monoclonal antibody 741F8 were examined in scid mice bearing SK-OV-3 tumor xenografts. 741F8 **sFv** monomers exhibited rapid, biphasic clearance from blood, while a slightly slower clearance was observed with the divalent 741F8 (**sFv**')2 comprising a pair of 741F8 **sFv**' with a C-terminal Gly4Cys joined by a disulfide bond. Following i.v. injection, the 741F8 **sFv** monomer was selectively retained in c-erbB-2-overexpressing SK-OV-3 tumor, with excellent tumor:normal organ ratios uniformly exceeding 10:1 by 24 h. The specificity of this effect was demonstrated by the lack of retention of the anti-digoxin 26-10 **sFv** monomer, as evaluated by biodistribution studies, gamma camera imaging, and cryomacroautoradiography studies. The specificity index (741F8 **sFv** retention/26-10 **sFv** retention) of 741F8 monomer binding, measured by the percentage of injected dose per g of tissue, was 13.2:1 for tumor, and 0.8 to 2.1 for all tested normal organs, with specificity indices for tumor:organ ratios ranging from 7.0 (kidneys) to 16.7 (intestines). Comparing divalent 741F8 (**sFv**')2 with the 26-10 (**sFv**')2, similar patterns emerged, with specificity indices for retention in tumor of 16.9 for the Gly4Cys-linked (**sFv**')2. These data demonstrate that, following their i.v. administration, both monovalent and divalent forms of 741F8 **sFv** are specifically retained by SK-OV-3 tumors. This antigen-specific binding, in conjunction with the 26-10 **sFv** controls, precludes the possibility that passive diffusion and pooling in the tumor interstitium contributes significantly to long-term tumor localization. 741F8 (**sFv**')2 species with peptide **spacers** exhibited divalent binding and increased retention in tumors as compared with 741F8 **sFv** monomers. Since the blood retention of the (**sFv**')2 is slightly more prolonged than that of the monomer, it was necessary to demonstrate that the increased tumor localization of the peptide-linked (**sFv**')2 was due to its divalent nature. The significantly greater localization of the divalent bismalimidohexane-linked 741F8 (**sFv**')2 as

compared with a monovalent 741F8 Fab fragment of approximately the same size suggests that the increased avidity of the (sFv')2 is a factor in its improved tumor retention. This is the first report of successful specific in vivo targeting of tumors by divalent forms of sFv molecules. The improved retention of specific divalent (sFv')2 by tumors may have important consequences for targeted diagnostic or therapeutic strategies.

WEST

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L18: Entry 12 of 13 File: DWPI Apr 1, 2000

DERWENT-ACC-NO: 1992-234646
DERWENT-WEEK: 200124

TITLE: New chimeric receptor proteins activate sec. messenger pathways - useful for activating leukocytes in response to ligands associated with e.g. viral infections and cancers

INVENTOR: CAPON, D J; IRVING, B A ; WEISS, A

PRIORITY-DATA: 1990US-0627643 (December 14, 1990)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
KR 246529 B1	April 1, 2000		000	C12Q001/68
WO 9210591 A1	June 25, 1992	E	054	C12Q001/68
AU 9191722 A	July 8, 1992		000	C12Q001/68
EP 517895 A1	December 16, 1992	E	054	C12N015/62
NO 9203171 A	September 28, 1992		000	C07K015/00
JP 05504262 W	July 8, 1993		054	C12N015/62
AU 643109 B	November 4, 1993		000	C12N015/11
EP 517895 A4	September 1, 1993		000	C12Q001/68
EP 732402 A2	September 18, 1996	E	014	C12N015/62
EP 517895 B1	November 20, 1996	E	018	C12N015/62
DE 69123241 E	January 2, 1997		000	C12N015/62
ES 2096749 T3	March 16, 1997		000	C12N015/62
EP 732402 A3	May 21, 1997		000	C12Q001/68
NO 308618 B1	October 2, 2000		000	C12N015/62

INT-CL (IPC): A01N 63/00; C07H 15/12; C07H 17/00; C07K 3/00; C07K 13/00; C07K 14/00; C07K 14/73; C07K 14/74; C07K 15/00 ; C07K 19/00; C12N 5/00; C12N 5/08; C12N 5/10; C12N 5/22; C12N 15/11; C12N 15/12; C12N 15/62; C12N 15/63; C12P 21/00; C12P 21/06; C12Q 1/68; G01N 33/53

ABSTRACTED-PUB-NO: EP 517895B
BASIC-ABSTRACT:

A novel DNA sequence (I) comprises, in reading frame: (a) a sequence encoding a signal sequence; (b) a sequence encoding an extracellular surface membrane protein domain binding specifically to at least one ligand (alone or with a second extracellular domain); (c) a trans membrane domain-encoding sequence; and (d) a sequence encoding a cytoplasmic domain of the T-cell receptor (TCR)-associated zeta chain or eta chain, or gamma chain of the FcepsilonR1 receptor. The extracellular domain and the cytoplasmic domain are not naturally joined together.

USE/ADVANTAGE - The chimeric proteins have a cytoplasmic region associated with transduction of a signal and activation of a secondary messenger system, e.g. involving a kinase, and an extracellular region capable of binding to a specific ligand and transmitting to the cytoplasmic region a signal (on formation of a

binding complex). In this way, cells capable of expressing the chimeric protein can be activated by contact with the ligand. This allows modulation of cellular responses with ligands other than that normally associated with activation, where for some reason the natural agent may not be useful. For example, T cells, which are useful as cytotoxic agents or as activating agents themselves, are restricted in their activation; the ability of the T cell receptor to recognise antigen is dependent on the nature of the host MHC molecules. Use of a chimeric T-cell receptor, having a non-MHC restricted ligand binding domain would permit the use of the engineered effector T-cell in any individual, regardless of MHC genetic background. By using CDA as the extracellular domain, binding of an HIV protein would result in activation of a T-cell, to destroy infected cells
ABSTRACTED-PUB-NO:

WO 9210591A EQUIVALENT-ABSTRACTS:

A chimeric DNA sequence encoding a membrane bound protein, said DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding an extracellular binding domain of a surface membrane protein that binds specifically to at least one ligand, wherein ligand is a protein on the surface of a cell or a viral protein; a sequence encoding a trans membrane domain; and a sequence encoding a cytoplasmic domain of a protein capable of transmitting a signal wherein said cytoplasmic domain is the eta or zeta domain of the T cell receptor or the gamma chain of the Fc(epsilon)R1 receptor, wherein said extracellular domain and cytoplasmic domain are not naturally joined together and said cytoplasmic domain is not naturally joined to an extracellular ligand-binding domain, and when said chimeric DNA sequence is expressed as a membrane bound protein in a selected host cell under conditions suitable for expression, the binding of a ligand to the extracellular domain leads to transmission of a signal to the cytoplasmic domain, resulting in activation of a signalling pathway in host cell.

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L16: Entry 2 of 11 File: USPT Mar 13, 2001

DOCUMENT-IDENTIFIER: US 6200801 B1
TITLE: Serpin enzyme complex receptor-mediated gene transfer

PRAD:
19950323

DEPR:
The present invention addresses two of the problems associated with effective gene therapy: the immunogenicity (i.e., antigenicity) and molecular heterogeneity of the gene transfer complex. Thus, to improve a very promising strategy of gene therapy, the immunogenicity of the gene transfer complex is minimized or abolished by constructing chimeric monoclonal antibodies and single-chain antibodies. As the heterologous sequences in the Fabs are the most likely cause of the immune response, most of the heterologous sequences in the Fabs are replaced with same-species sequences (i.e., for use in humans, chimeric rodent-human monoclonals are generated). To further reduce the immunogenicity of the gene transfer complex, single chain antibodies were generated. The molecular heterogeneity of the complex is reduced or abolished by using monoclonal antibodies directed against human SC, and by constructing fusion proteins comprising a single-chain Fv molecule, directed against the pIgR, in a fusion protein with a polycation. These fusion proteins obviate the need to chemically couple a polycation to the Fab.

DEPR:
Acute disorders might also be ameliorated by direct delivery of genes to airway epithelium using the methods of the present invention. For example, patients who must be treated with high FIO.sub.2 or patients with the acute respiratory distress syndrome (ARDS) would benefit from high levels of superoxide dismutase expression in the airways. Patients about to undergo chemotherapy with bleomycin might have their lungs protected against dose-limiting toxicity by transfer of bleomycin hydroxylase to the lung. Treatments for acute stresses may require only transient gene expression. Moreover, the use of antibody-mediated receptor-targeted gene therapy directed at receptors other than the pIgR will allow the treatment of a variety of disorders. The methods described below, while illustrated using the pIgR system, are generally applicable for the development of antibodies, including single-chain antibodies, against receptors other than the pIgR.

DEPR:
Chimeric rodent/human MAbs (prepared as described in Example 8) contain the variable domain and antigen binding characteristics of rodent origin but utilize the human constant domains, the domains most likely to be immunogenic. These chimeric MAbs should be less immunogenic when administered to a human than the corresponding Fab fragments isolated from the rodent MAb. To further reduce the potential immunogenicity of antibodies capable of binding to the human pIgR, single-chain antibody molecules (scFvs) directed against human SC (pIgR) were generated. The scFvs generated include scFv alone and scFv as a fusion protein with mouse protamine sequences. The scFv fusion proteins which contain polycation sequences (e.g., protamine) avoid the need to chemically couple a polycation to the antibody used to target DNA to the pIgR.

DEPR:
Single-chain antibodies consist of an antibody light chain variable domain

(V.sub.L) and heavy chain variable domain (V.sub.H) connected by a short peptide linker. The peptide linker allows the structure to assume a conformation which is capable of binding to antigen [Bird et al., (1988) Science 242:423 and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879]. Single-chain antibodies directed against cell surface proteins (e.g., transferrin receptor, the p55 subunit of the interleukin 2 receptor) have been used as one portion of a fusion protein with a cytotoxin (e.g., Pseudomonas exotoxin A or diphtheria toxin) [Batra et al. (1991) Mol. Cell. Biol. 11:2200; Chaudhary et al. (1989) Nature 339:394; Chaudhary et al. (1990) Proc. Natl. Acad. Sci. USA 87:949; Pantoliano et al. (1991) Biochem. 30:10118]. These fusion proteins kill only those cells which express the appropriate receptor, and some are up to 750 times more potent than their congeners assembled from the components by chemical means [Batra et al. (1991), supra; and Chaudhary et al. (1989) Nature, supra]. This improvement in efficiency occurs despite a loss of 2-10 fold in binding efficiency of the Fv compared to native antibody from which it was derived. Presumably the poorer affinity of the Fv is compensated by less distortion of the binding site in the course of the chemical coupling.

DEPR:
The single-chain Fv construct (as a HindIII-XbaI fragment) was subdloned into pRC/CMV (Invitrogen) which had been digested with HindIII and XbaI sites. The resulting construct was termed pRC/CMV-4121 scFv.

DEPR:
RNA was extracted from mouse testes and was reversed transcribed into cDNA by Moloney murine leukemia virus reverse transcriptase using random hexamers as primers. The protamine cDNA was then amplified by PCR using Primers 5 and 6. The resulting PCR product was digested with ClaI and XbaI and was subcloned into pRC/CMV-4121 scFv which had been digested with ClaI and XbaI to generate the fusion construct. The fusion construct was termed pRC/CMV-4121 scFv/protanine. The DNA sequences encoding the anti-human SC single-chain Fv/protamine fusion protein are listed in SEQ ID NO:24. The amino acid sequence of the anti-human SC single-chain Fv/protamine fusion protein is listed in SEQ ID NO:25.

DEPR:
Analysis of the DNA sequence encoding the single-chain Fv protein revealed that a single codon for glycine located within the linker region had been dropped during the assembly of the single-chain Fv. The resulting single-chain Fv contains a linker having the amino acid sequence GGGGSGGGGSGGGS (SEQ ID NO:26) instead of the intended (GGGGS).sub.3 (SEQ ID NO:27). The linker region in a single-chain Fv is reported to require a length of 3.5 .ANG. or greater (3.5 .ANG. being the distance between the light and heavy chain variable regions in native antibodies as determined by crystal structure) [Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879]. As each peptide bond is 0.38 .ANG. in length, the 14 amino acid linker present in the anti-human SC single-chain Fv would more than satisfy the linker length requirement reported by Huston et al. Pantoliano et al. reported that the affinity of the single-chain Fv is reduced when the linker is only 12 amino acids in length but that lengths between 14 and 25 amino acids show similar binding affinity to the target protein [Biochem. (1991) 30:10117]. The 4121 single-chain Fv containing the 14 residue linker has been shown to recognize the receptor protein by "Western blot" type analysis (described below).

DEPC:
Production of Anti-Human SC Single-Chain Antibodies

L14: Entry 33 of 69 File: USPT Apr 7, 1998

DOCUMENT-IDENTIFIER: US 5736387 A
TITLE: Envelope fusion vectors for use in gene delivery

APD:
19940601

BSPR:
There have been several attempts to confer specificity to retroviral infection. Russell et al. linked a single-chain antibody molecule to a retroviral envelope protein (Nucleic Acids Res 5:1081, 1993). The resulting protein was able to promote binding of viral particles to plastic dishes coated with the appropriate antibody; but binding to cells was not tested. It was unclear, therefore, whether such complexes could effectively bind to cells and, even if they could bind, whether they could also mediate viral uptake. Goud et al. attempted to link ecotropic Moloney murine leukemia virus (MoMLV) to human cells using an anti-(env) antibody and an anti-(human transferrin receptor) antibody (Virology 163:251, 1988). The two antibodies were linked by a third antibody directed against an immunoglobulin light chain determinant. Although it appeared that some internalization of the complex occurred using this "bridging" approach, there was no active infection. In another approach using antibody derivatives, Roux et al. linked an anti-(env) antibody to an antibody (W6/32) directed against an HLA framework epitope (PNAS 86:9079, 1989). In this study, some infection of human HeLa cells by the conjugates was achieved. Although such conjugates were also shown to work for targeting through a cytokine receptor, namely the EGF receptor, this approach did not appear to be generally applicable since a number of antibody conjugates did not work at all and, in those which exhibited some infectivity, the efficiency of targeting was reported to be quite low (Etienne-Julan et al., J. Gen. Virol., 73:3251-55, 1992). Neda et al. reported some infectivity of human hepatic cells, apparently via the galactose receptor, using viral particles with lactose on their surface (J. Biol. Chem. 266:14143-14146, 1991). Another indirect "bridging" approach attempted to exploit the known ability of lectins to bind glycoproteins by providing a variety of multivalent lectins which might bind, and therefore bridge, glyco-conjugates on the cellular surface and those on the retroviral surface (Etienne-Julan, id.). Although binding was apparently detected, there was no observable infection of the target cells using such complexes.

BSPR:
Accordingly, one embodiment of the invention is a chimeric targeting protein (or "CTP") comprised of a ligand moiety and an uptake moiety, wherein the ligand moiety is an analog of a cytokine and is capable of binding to a cognate cytokine receptor present on the surface of a target cell, and wherein the uptake moiety is an analog of a viral envelope protein and is capable of promoting entry of the vector into the target cell. In a first type of CTP, the ligand moiety also exhibits "cytokine effector activity" (as defined herein), which can be used to modulate the growth, differentiation or other activity of the targeted cell. In a second type of CTP, the ligand moiety does not exhibit cytokine effector activity. In a preferred embodiment, the uptake moiety has a reduced ability (i.e. a completely or partially reduced ability) to bind to the natural env receptors but retains the ability to promote viral uptake. In another preferred embodiment, the ligand moiety and the uptake moiety are separated by a flexible peptide linker sequence, or "flexon," which is believed to enhance the ability of the moieties to adopt conformations relatively independently of each other.

DEPR:
In another preferred embodiment, the ligand moiety and the uptake moiety are separated by a flexible peptide linker sequence, or "flexon," which is believed to enhance the ability of the moieties to adopt conformations relatively independently of each other.

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L14: Entry 29 of 69 File: USPT Jun 9, 1998

DOCUMENT-IDENTIFIER: US 5763733 A
TITLE: Antigen-binding fusion proteins

APD:
19941013

BSPR:
Fusion proteins consisting of a single-chain antibody fused to interleukin-2 have been reported (Savage, P., The Third Annual IBC International Conference on Antibody Engineering: New Technology and Application Implications, International Business Communications, Southborough, Mass. (1992)).

DRPR:
FIG. 7 depicts a competition radioimmunoassay of CC49 IgG (.sup.125 I labeled). CC49 IgG is competed against PLAP No. 1, PLAP No. 2 and CC49/218 SCA (labeled SFv CC49 218). PLAP No. 1 and PLAP No. 2 represent two different preparations of CC49/212 SCA with PLAP C-terminus.

DRPR:
FIG. 9A depicts an SCA-TNF construct (SEQ ID NOS: 12-15) where the two amino terminal amino acid residues are removed from a genetically modified form of TNF and the truncated TNF is fused to CC49 sFv.

DEPR:
In some cases it may be necessary to separate the antigen-binding part of a fusion protein from the immunoeffector of cytolytic part of the fusion protein by a peptide spacer, in order to preserve both activities of the fusion protein. It is preferred that the spacers are between 0 and 50 amino acids in length.

DEPR:
Moreover, the PLAP single-chain antibody fusion proteins will be able to bind certain tumors and activate a localized immune response to the tumors.

DEPR:
Another preferred construct has the immunoeffector or cytolytic region of TNF, or the TNF sequence beginning with NH.sub.2 -Arg Ser Ser Ser Arg Thr Pro Ser Asp . . . fused to the carboxyl terminus of a CC49 sFv construct, particularly a construct with a 2-mer or 10-mer spacer (see FIG. 9A-B (SEQ ID NOS: 12-16), for example).

ORPL:
Dorai et al., "Mammalian Cell Expression of Single-Chain Fv (sFv) Antibody Proteins and Their C-terminal Fusions with Interleukin-2 and Other Effector Domains," Bio/Technology 12:890-897 (Sep. 1994).

ORPL:
Savage et al., "A recombinant single chain antibody interleukin-2 fusion protein," Br. J. Cancer 67:304-310 (1993).

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Search Results - Record(s) 1 through 7 of 7 returned.

☐ 1. Document ID: US 6004811 A

L5: Entry 1 of 7 File: USPT Dec 21, 1999

US-PAT-NO: 6004811
DOCUMENT-IDENTIFIER: US 6004811 A
TITLE: Redirection of cellular immunity by protein tyrosine kinase chimeras

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWMC | Draw. Desc | Image

☐ 2. Document ID: US 5912170 A

L5: Entry 2 of 7 File: USPT Jun 15, 1999

US-PAT-NO: 5912170
DOCUMENT-IDENTIFIER: US 5912170 A
TITLE: Redirection of cellular immunity by protein-tyrosine kinase chimeras

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWMC | Draw. Desc | Image

☐ 3. Document ID: US 5888773 A

L5: Entry 3 of 7 File: USPT Mar 30, 1999

US-PAT-NO: 5888773
DOCUMENT-IDENTIFIER: US 5888773 A
TITLE: Method of producing single-chain Fv molecules

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWMC | Draw. Desc | Image

☐ 4. Document ID: US 5830755 A

L5: Entry 4 of 7 File: USPT Nov 3, 1998

US-PAT-NO: 5830755
DOCUMENT-IDENTIFIER: US 5830755 A
TITLE: T-cell receptors and their use in therapeutic and diagnostic methods

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWMC | Draw. Desc | Image

☐ 5. Document ID: US 5798100 A

L5: Entry 5 of 7 File: USPT Aug 25, 1998

US-PAT-NO: 5798100
DOCUMENT-IDENTIFIER: US 5798100 A
TITLE: Multi-stage cascade boosting vaccine

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw. Desc | Image

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☐ 6. Document ID: US 5712149 A

L5: Entry 6 of 7 File: USPT Jan 27, 1998

US-PAT-NO: 5712149
DOCUMENT-IDENTIFIER: US 5712149 A
TITLE: Chimeric receptor molecules for delivery of co-stimulatory signals

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw. Desc | Image

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☐ 7. Document ID: US 5359046 A

L5: Entry 7 of 7 File: USPT Oct 25, 1994

US-PAT-NO: 5359046
DOCUMENT-IDENTIFIER: US 5359046 A
TITLE: Chimeric chains for receptor-associated signal transduction pathways

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw. Desc | Image

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